Induced Pluripotent Stem Cell-Derived Retinal Pigmented Epithelium: A Comparative Study Between Cell Lines and Differentiation Methods.

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Public Summary:
This study provides a comparison between 2 different methods for RPE differentiation: (1) a commonly used spontaneous continuously adherent culture (SCAC) protocol and (2) a more rapid, directed differentiation using growth factors.

Scientific Abstract:
PURPOSE: The application of induced pluripotent stem cell-derived retinal pigmented epithelium (iPSC-RPE) in patients with retinal degenerative disease is making headway toward the clinic, with clinical trials already underway. Multiple groups have developed methods for RPE differentiation from pluripotent cells, but previous studies have shown variability in iPSC propensity to differentiate into RPE. METHODS: This study provides a comparison between 2 different methods for RPE differentiation: (1) a commonly used spontaneous continuously adherent culture (SCAC) protocol and (2) a more rapid, directed differentiation using growth factors. Integration-free iPSC lines were differentiated to RPE, which were characterized with respect to global gene expression, expression of RPE markers, and cellular function. RESULTS: We found that all 5 iPSC lines (iPSC-1, iPSC-2, iPSC-3, iPSC-4, and iPSC-12) generated RPE using the directed differentiation protocol; however, 2 of the 5 iPSC lines (iPSC-4 and iPSC-12) did not yield RPE using the SCAC method. Both methods can yield bona fide RPE that expresses signature RPE genes and carry out RPE functions, and are similar, but not identical to fetal RPE. No differences between methods were detected in transcript levels, protein localization, or functional analyses between iPSC-1-RPE, iPSC-2-RPE, and iPSC-3-RPE. Directed iPSC-3-RPE showed enhanced transcript levels of RPE65 compared to directed iPSC-2-RPE and increased BEST1 expression and pigment epithelium-derived factor (PEDF) secretion compared to directed iPSC-1-RPE. In addition, SCAC iPSC-3-RPE secreted more PEDF than SCAC iPSC-1-RPE. CONCLUSIONS: The directed protocol is a more reliable method for differentiating RPE from various pluripotent sources and some iPSC lines are more amenable to RPE differentiation.